AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 29, NUMBER 2, JUNE 2000

EDITOR:
B. O. OSOTIMEHIN

ASSISTANT EDITOR:
A. O. UWAIFO

ISSN 1116 — 4077

Afr. J. Med. med. Sci. (2000) 29, 141-144

Seasonal variations of hydroxyanthraquinone content of cultivated *Cassia spectabilis* DC

KA. Abo, *AA. Adeyemi and +AO. Sobowale

*Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Ibadan, Ibadan, *Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo College of Health Sciences, Ogun State University, Sagamu, +Department of Biological Sciences, Ogun State University, Ago-Iwoye, Nigeria.*

Summary

Seasonal variations and spectrophotometric estimation of the anthraquinone content of cultivated *Cassia spectabilis* DC are presented. Concentrations of anthraquinones peaked in the leaves (1.03%) at flowering Significant ($P < 0.05$) variations in anthraquinone content were observed in the cultivated species. Anthraquinones reached peak levels during the months of September to January (dry season). There was a significant ($P < 0.05$) drop in anthraquinone content during the rainy season. The diagnostic microscopic features of this little studied species are also described. This study has provided vital information on the best period of harvest and seasonal distribution of anthraquinones in the leaves of the species.

Keywords: Cassia spectabilis, *microscopy, hydroxyanthraquinones, seasonal variations.*

Résumé

Les variations saisonieres et les estimations spectrophotouremetric du contenue de l'anthraquinone dons les culturesde *cassia spectabilis* DC cont presente's Les concentrations de l'anthraquinones avasent atteint leus pe maximum (1.03%) dons les facilles pendant la floraison. Less variations significative ($P < 0.05$) dours le contenue d'anthraquinoe avait etait obserce dous les especes culture's. Le taux d'anthraquinone avait atteind son emaximum pendant les evois de septembre a Januvier (faison feche). I'l yavait une reduction significative. Sur le contenue d'anthraquinone pendant la raison de pluire. Le diagnostique microscopique de l'apparence des fecilles dons cette petite etude est aussi decrite. Cette etude a fournit des informations vitales. Sur la meillure des periode de recolte et la distribution saisoniere de l'anthraquinone dons les fenelles des especes etudies.

Introduction

Cassia spectabilis DC (Leguminosae – Caesalpinioideae) is one of the 33 *Cassia* species growing in Nigeria [1]. It is used in traditional medicine for the treatment of venereal and skin diseases and as an abortifacient [2,3].

Correspondence: Dr. K.A. Abo, Dept of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan Nigeria.

C. spectabilis growing in Argenitina has been shown to contain 1, 8-dihydroxy-3-methoxyanthraquinone (physcion) and 1,3,8-trihydroxy-2-methylanthraquinone [4] which are established anthraquinone purgatives. The methanolic extract of this species has also been shown to exhibit antibacterial activity against *B. subtilis, S. aureus, S. lutea, S. albus, M. tuberculosis and V. coma* [4].

Our interest in this species arose because very little is known of the Nigerian variety which is used by herbalists in Ibadan. Recent studies in our laboratory show that the Nigerian variety exhibit potent dose related laxative and antibacterial activity [5]. Field survey revealed that *C. spectabilis* is often confused with *C sieberiana* by the indigenous population because of their very close morphological features. This study describes, for the first time, the characteristic microscopic features of the leaf of *C. spectabilis*.

Furthermore, significant variations in hydroxyanthraquinone content have been recorded in some *Cassia* species growing in Nigeria [5-7]. The main objective of this work is to investigate the effect of seasonal changes on the accummulation of anthraquinone glycosides and water soluble extractive values of *C. spectabilis* DC cultivated in Ibadan.

Materials and methods

Plant material: Morphological parts of *C. spectabilis* DC were collected in February 1997 from a cultivated species in Ibadan and authenticated at the Forestry Research Institute of Nigeria, Ibadan, where a herbarium specimen is kept. Fresh organs were collected in the mornings and for seasonal variation studies, leaves were harvested weekly over a period of one year. Samples were dried at 45°C, powdered and stored in sealed amber glass containers until ready for analysis. Five replicate determinations were performed on each batch.

Microscopy: The powdered sample of the leaves was sieved and the collected fine powder was cleared over a bunsen flame or bleached by soaking in chloral hydrate solution B.P. for 2-4 days. This was subsequently stained with phloroglucinol/conc. HCl, mounted in glycerol and examined under the Olympus binocular research microscope.

Transverse sections of the midrib and lamina of the leaf were prepared for microscopic examination as described in the African Pharmacopoeia [8]. The results are shown in Figures 1-3.

Identification test: The presence of anthraquinone glycosides in the batches was ascertained following extraction into chloroform and detection by the Borntrager reaction as previously described [9].

Determination of water soluble extractive value: Water soluble extractive values were determined on 4.0 gm of the air-dried powdered sample as described in the African Pharmacopoeia [8].

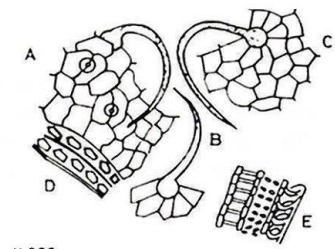
Spectrophotometric estimation of total anthraquinone content.

The standard procedure for the determination of anthraquinone content of *Cassia* species was adopted [5,7,10]. About 500 mg of defatted powdered sample of *C. spectabilis* was refluxed with 50mls of 20% methanol for 15 min. The extract was filtered and made up to 50 ~~mls~~ with 20% methanol. 10 ml was taken and further refluxed with 1ml of concentrated hydrochloric acid and 2 ml of 20% (W/V) ferric chloride solution for 15 min. The cooled reaction mixture was partitioned into chloroform (2 x 30 mls), made to 100 mls with fresh chloroform and dried over anhydrous sodium sulphate. In all 10 mls of the diluted extract was evaporated to dryness, cooled and reconstituted to 10 ml with 1M potassium hydroxide solution. The absorbance of the solution was immediately measured at 500 nm in a 1 cm cell using a Gallenkamp SPR-500 uv spectrophotometer. The assay was verified by comparison with data obtained from a linear calibration curve derived from dilutions of 1,8-dihydroxyanthraquinone (Sandoz, London).

Results and discussion

The cultivated species is a tree made up of compound leaves with about four to five pairs of leaflets arranged in opposite direction. The flowers are yellow while the pods are thin, flat with pointed ends. It also has ridges separating the seed compartments. The powdered leaf sample was characterised by straight-walled epidermal cells exhibiting numerous unicellular, finely warty appressed trichomes. The xylem cells were lignified and of the spiral, reticulate and annular types. The stomata were more evident on the lower epidermis and were of the paracytic type. Calcium oxalate (prisms) sheaths were observed around the vascular bundles (Figure 1).

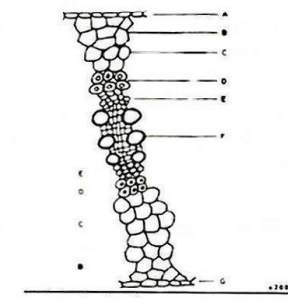
The transverse section of the leaf of *C. spectabilis* (Figure 2) showed a thin smooth cuticle with occasional unicellular appressed covering trichomes. The upper epidermis consist of a single layer of cells. Below the epidermis were numerous angular collenchyma followed by layers of somewhat smaller parenchyma cells. The vascular bundle is surrounded by rows of thick-walled



X 200

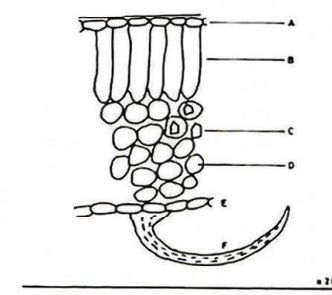
A = Lower epidermis with paracytic stomata;
B = Unicellular covering trichome
C = Upper epidermis
D = Cal Ox Sheath
E = Lignified xylem

Fig. 1: Microscopic features of powdered Cassia spectabilis leaves



A = Upper epidermis, B = Collenchyma, C = Parenchyma, D = Pericyclic fibers, E = Phloem, F = Xylem, G = Lower epidermis

Fig. 2: Transverse section through the midrib of the leaf of Cassia *spectabilis*



A = Upper epidermis, B = Palisade cells, C = Calcium oxalate prism, D = Spongy mesophyll, E = Lower epidermis, F = Unicellular trichome

Fig. 3: Transverse section through the lamina of the leaf of Cassia spectabilis

pericyclic fibres. The xylem vessels were lignified. The transverse section of the lamina (Figure 3) shows only one layer of closely arranged columnar palisade cells present only on the upper surface. The spongy mesophyll consists of loosely arranged cells with large intercellular spaces which sometimes contain prisms of calcium oxalate crystals. A few unicellular appressed trichome were found on the lower epidermis

The rose pink colour observed in the Borntrager test confirmed the presence of anthraquinone glycosides in the batches of *C. spectabilis*. Table 1 shows the period of growth of the plant when accumulation of anthraquinones peaked in the different morphological parts of the cultivated species. It shows that anthraquinones are concentrated in the leaves (1.03%) at peak flowering.

Table 1: Total anthraquinone content of organs of cultivated *Cassia spectabilsi*

Plant Organ	Period of harvest	Total anthraquinone Contents (% w/v)*
Leaf	Peak flowering	1.03 ± 0.31
Flower	Peak flowering	0.34 ± 0.42
Bark	Peak fruiting	0.17 ± 0.19
Pod	Maturity (before Fruit Dehiscence	0.28 ± 0.15

Values are mean ± S.D., n = 5
**Calculated as 1, 8-dihydroxyanthraquinone*

Table 2 shows seasonal distribution of anthraquinones in the leaves. The figure for each month is the mean (± S.D) of the appropriate weekly determinations. Anthraquinones reached peak levels during the months of September to January (dry season), the maximum being recorded in September and October. There was however significant drop ($P < 0.05$) in anthraquinone content at the onset of the rainy season and the level remained low during most parts of the rainy season. This finding suggests that rains may considerably lower the accummulation of some plant secondary metabolites since this finding is consistent with earlier study on the variations of alkaloid content in various *Datura* species [11–14]. On the contrary, the water soluble extractives peaked during the rainy season (Table 2).

This study has provided basic information on the best period of harvest and on the seasonal distribution of anthraquinones in leaves of *C. spectabilis*. This study partly explains some of the inconsistencies recorded in the anthraquinone content of some *Cassia* species growing in tropical countries [5 – 7].

Acknowledgements
Dr. K.A. Abo is grateful to the University of Ibadan for a Senate Research Grant. The authors are also grateful to staff of the Climatological Centre of the Department of Geography, University of Ibadan for providing the 1997/98 rainfall data.

Table 2: Seasonal distribution of total leaf anthraquinones of *Cassia specitabilis*

Month of Harvest	Average rainfall (mm/day)	Water soluble Extractive value	Total anthraquinone Content (% w/v) *
January	0.0	20.0 ± 1.46	0.71 ± 0.05
February	1.8	20.2 ± 0.05	0.25 ± 0.14
March	1.1	30.4 ± 0.00	0.04 ± 0.02
April	1.3	30.1 ± 0.05	0.29 ± 0.05
May	3.2	30.8 ± 0.00	0.33 ± 0.02
June	11.6	25.5 ± 0.07	0.21 ± 0.12
July	3.4	20.0 ± 0.00	0.14 ± 0.00
August	3.8	20.3 ± 0.00	0.29 ± 0.17
September	5.4	15.5 ± 0.21	1.46 ± 0.81
October	5.3	15.7 ± 0.23	1.63 ± 0.34
November	1.8	15.0 ± 0.24	0.46 ± 0.06
December	0.0	16.2 ± 0.27	0.92 ± 0.25

Values are mean ± S.D., n = 5
**Calculated as 1, 8-dihydroxyanthraquinone.*

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